

木霉菌株分离筛选及对茄子黄萎病防治作用的研究

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摘要:茄子黄萎病分布广泛,发病率一般为 50% 以上,是生产上的一种重要病害。为初步检测筛选的木霉菌株对茄子黄萎病的防治效果,在茄子嫁接、连作 13 a 的茄子病圃及轮作田的根际土壤进行木霉菌株的分离筛选,得到 15 株菌株,筛选出 2 株拮抗效果较强的菌株 M113 和 M135,抑菌率分别为 48.36% 和 56.13%。室内盆栽试验相对防效为 70.53% 和 74.68%,显著高于化学药剂防治。

关键词:生物防治;木霉;黄萎病

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茄子黄萎病是由大丽轮枝菌(*Verticillium dahliae*)引起的一种土传的维管束病害,被称为茄子的癌症^[1]。发病时植株叶片枯萎,蔓延速度快,严重时整株枯死,果实商品性差。茄子品种中可利用的种质资源中没有高抗材料,中抗材料仅占 0.4% 左右,主要是野生种和近缘种,茄子抗病育种工作难度极大。目前生产上主要以嫁接和轮作等栽培技术预防、抵御茄子黄萎病。

生物防治是利用有益生物或其它生物来抑制或消灭有害生物的一种防治方法,由于其具有不污染环境,对人们身体无伤害等优点,受到人们广泛的关注与应用,近年来成为研究热点。本研究在嫁接茄子植株、合理轮作茄子植株及黄萎病发生严重

病圃的茄子根际土壤分离木霉菌株,进行对峙培养试验、温室盆栽试验及田间小区的防效试验,初步检测筛选木霉菌株的防治效果。

1 材料与方法

1.1 材料

供试菌株为大丽轮枝菌,供试茄子品种为龙杂茄 7 号。

1.2 方法

试验于 2012 年在黑龙江省农业科学院园艺分院试验地进行。

1.2.1 木霉菌株的分离及鉴定 采集嫁接植株、合理轮作植株以及黄萎病发生严重病圃上的根际土壤,采用稀释平板法分离木霉菌株,纯化后根据 Rifai^[2]的方法对各个菌株进行分类鉴定。

1.2.2 木霉拮抗菌株的筛选 采用平板对峙试验,测定各木霉菌株对大丽轮枝菌的抑菌率,以只接病原菌不接木霉菌为对照,筛选对致病菌生长抑制最强的木霉菌株。

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Effect of Ecological Factors on the Performance of Biofilm System of Modified Polyurethane Filler

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Abstract: Carrier is the dominant factor during biofilm process for waste water treatment, in order to improve the operation efficiency of biofilm by ecological factors, taking the modified polyurethane filler (MPU) as research object, the effect of ecological factors on the performance of biofilm system was studied. The results showed that effluent COD and ammonia removal rate of MPU biofilm reactor achieved 70% after 10 days of operation, and formed a stable biofilm. The effluent COD and ammonia removal rate of biofilm system increased while enhancing the operation temperature. The effluent COD and ammonia removal rate achieved 80% while operating at 15℃. While the influent COD enhanced from 100 mg·L⁻¹ to 300 mg·L⁻¹, effluent COD removal rate increased from 60% to 80%, and the removal rate of ammonia nitrogen achieved 85%. In addition the effluent COD and ammonia removal rate increased while prolonged the HRT of biofilm system. The removal rate of COD increased from 80% to 90% and the removal rate of ammonia nitrogen reached 96% while prolonged HRT from 2 h to 8 h.

Keywords: waste water; biofilm; modified polyurethane filter; ecological factor

1.2.3 木霉拮抗菌的制备 将木霉菌在 PDA 平板培养基上培养 3 d,打直径为 5 mm 的菌碟 5 块,接种在每瓶 100 mL 的 PD 培养液中,将 pH 调至 6,25℃下 200 r·min⁻¹ 震荡培养 7 d,发酵液经滤纸过滤,滤液为含孢子发酵液。含孢子滤液通过细菌过滤器灭菌,即为无菌发酵滤液^[3],盆栽试验备用。

1.2.4 大丽轮枝菌接种体的制备 挑 10 块培养 4~8 d 的茄子黄萎病菌碟转入直径 90 mm 的培养皿内,加入蒸馏水至没过菌碟表面,每 30 min 换水 1 次,换水 4 次后加入 15 mL Petri 培养液,25℃条件下培养 18~20 h,盆栽试验采用灌根处理。

将活化后的病原菌菌丝块接种到灭菌后的玉米砂培养基中,20~25℃下培养 30 d,长满菌丝后,选无杂菌的培养物作为田间病原物接种体^[4]。

2 结果与分析

2.1 木霉分离鉴定结果

从连作 13 a 的茄子病圃田、嫁接茄子根际土壤及合理轮作茄子地块分离得到木霉菌株 15 株,经鉴定后为黄绿木霉、绿色木霉和哈茨木霉,其中以黄绿木霉为主,占 57.7%。

2.2 对峙培养法筛选拮抗木霉菌株

通过对土壤样品进行系列稀释涂布培养获得单菌落,纯化、培养。从获得的 15 株木霉菌株中筛选出拮抗效果较好的 2 株,分别为 M113 和 M135(见图 1)。木霉菌株 M113 拮抗效果显著,在两菌落中间形成了明显的黄色拮抗线,与对照

相比有效抑制了大丽轮枝菌的生长,抑菌圈直径达 22.00±2.32 mm,抑菌率达到 48.36%;木霉菌株 M135 不仅有效阻止了病原菌的生长,而且生防菌菌丝体生长到了致病菌内部,达到了寄生的效果,病原菌在平板培养基上生长缓慢,菌丝稀疏、长势较慢,抑菌率达到了 56.13%。14 d 后木霉菌菌丝体已经覆盖到茄子黄萎病致病菌的表面,进一步抑制致病菌的生长。

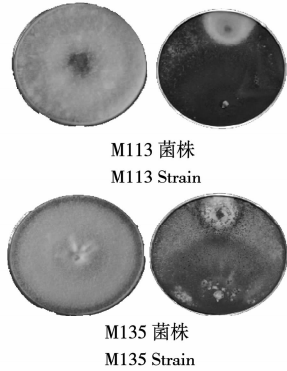


图 1 木霉拮抗菌株筛选试验

Fig. 1 Screening of *Trichoderma viride* strain

2.3 木霉拮抗菌株室内盆栽试验

木霉菌株浓度为 5×10⁷ 时,木霉拮抗菌株 M113 和 M135 病情指数分别为 26.27%和 22.57%,低于化学药剂多菌灵的 67.64%,防治效果也高于化学药剂的 24.11%,防效均在 70%以上。

表 1 木霉菌拮抗试验及对茄子黄萎病盆栽防效试验

Table 1 *Trichoderma* antagonism experiment and pot control effect of eggplant verticillium wilt

处理 Treatments	抑菌圈直径/mm Bacteriostatic circle diameter	抑菌率/% Bacteriostatic rate	病情指数/% Disease index	相对防效/% Relative control effect
M113	22±2.32	48.36	26.27 c	70.53 a
M135	27±1.55	56.13	22.57 c	74.68 a
0.3%多菌灵			67.64 b	24.11 b
0.3% Carbendazim				
清水(CK) Water			89.13 a	0 c

不同小写字母表示在 0.05 水平差异显著。下同。

Different lowercases mean significant difference at 0.05 level. The same below.

表 2 木霉拮抗菌株对茄子黄萎病田间抗病性鉴定

Table 2 Resistance identification of *Trichoderma viride* strain on eggplant *Verticillium* wilt

处理 Treatment	发病率/% Incidence	病情指数/% Disease index	相对防效/% Relative control effect
M113	40.26 b	32.14 b	33.71 b
M135	41.57 b	30.48 b	37.14 ab
0.3%多菌灵	40.69 b	28.16 b	41.93 a
0.3% Carbendazim			
清水(CK) Water	60.17 a	48.49 a	0 c

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Determination of Chlorogenic Acid in *Evodia lenticellata* Huang

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Abstract: In order to further develop and utilize *Evodia lenticellata* Huang resources, the chlorogenic acid were extracted from *E. lenticellata* Huang using 50% methanol, and the conditions of the ultrasonic wave as follows: extraction ultrasonic power of 240 W, ultrasonic frequency of 40 kHz, temperature of 60°C and ultrasonic treatment time of 30 min. Samples were analyzed on an Inertsil ODS-3C₁₈ column (4.6×150 mm, 5 μm) using acetonitrile-0.4% phosphoric acid (8:92) as mobile phase, speed of flow was 1.0 mL·min⁻¹, the examination wave length was 327 nm and the column temperature was 30°C. The chlorogenic acid showed a good linearity in the range of 20.8~104 μg·mL⁻¹, regression equation was $Y = 32\,304X - 44\,968$ ($R^2 = 0.999\,9$) and the average recovery was 99.59% (RSD=1.69%, n=6). The chlorogenic acid is richer in *E. lenticellata* Huang and there are some different in the content of chlorogenic acid in *E. lenticellata* Huang among different origins. The established method is simple, credible, accurate, repeatable and could be used for the quality control of *E. lenticellata* Huang.

Keywords: *Evodia lenticellata* Huang; chlorogenic acid; content determination

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2.4 木霉拮抗菌株田间防治效果试验

田间防效试验的施用时期为移苗期、定植期及定植后 14 d, 移苗期每钵施用 5 g 木霉培养物, 定植期及定植后施用 15 g 木霉培养物, 浓度选择为 5×10^7 个·g⁻¹, 于 8 月中旬发病高峰期调查各处理的病情指数。M113 菌株处理的发病率 40.26%, M135 发病率为 41.57%, 与 0.3% 多菌灵发病率 40.69% 相近。2 个菌株病情指数分别为 32.14% 和 30.48%, 均略高于化学药剂多菌灵的 28.16%。结果表明, M135 的相对防效高于 M113, 木霉菌株 M113 和 M135 在田间防效试验中没有显著差异, 但均低于 0.3% 多菌灵的 41.93%, 但差异不显著, 两个供试菌株均有较好的防治效果。

3 结论与讨论

木霉菌株 M113 和 M135 在实验室抑菌试验和盆栽试验阶段均有较好的防治效果, 显著高于多菌灵的防治效果。在田间防效试验略低于多菌

灵, 但差异不显著。木霉菌株在实验室阶段和田间试验阶段防治效果存在差异, 主要原因可能是受到自然条件、天气状况、土壤条件、木霉制剂在土壤中的生长状况及使用量等方面的影响和制约^[5], 防效效果表现不稳定。如何克服外界环境条件对生防菌的影响是生防菌最大发挥防效的关键所在, 也是下一步研究的重点。

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Study on Separation and Screening of *Trichoderma* Strain and Control Effect of *Verticillium* Wilt

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Abstract: Eggplant *Verticillium* wilt are widely distributed, the incidence are more than 50% commonly, it is a kind of important disease in eggplant production. In order to test the control effect of screening strains, the separation and screening of *Trichoderma* were conducted in the disease nursery for 13 years eggplant grafting and rotation field in rhizosphere soil, 15 strains were obtained, 2 strains with strong antagonism M113 and M135 were screened out, the bacteriostatic rate were 48.36% and 56.13% respectively. Indoor pot experiment results showed that the relative control effect were 70.53% and 74.68%, which were significantly higher than chemical prevention and control.

Keywords: biological control; *Trichoderma*; *Verticillium* wilt